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(54) IMPROVED MICROBIOLOGICAL TESTING DEVICE AND PROCESS FOR PREPARATION

(71) We, UNION CARBIDE CORPORATION, a corporation organised and existing under the laws of the State of New York 10017, United States of America, of 270, Park Avenue, New York, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to an, improved microbiological testing device and to a process for its preparation. In one aspect, this invention is directed to an improved testing device containing an insoluble hydrophilic gel integrally bonded thereto and which is useful for the propagation of bacteria, fungi, viruses, and tissues of more complex organisms. In a further aspect, this invention is directed to a process for the preparation of the improved microbiological testing devices and to a method for their use.

Heretofore, one of the most widely used methods for the propagation of microorganisms was to inoculate a nutrient mixture solidified with agar and contained in a Petri dish. Agar, over the years, has become the standard solid phase utilized in the art of microorganism propagation. This material is prepared from various species of Gelidium and closely related algae and is insoluble in cold water but soluble in hot water. A dilute, neutral aqueous solution of from about 1 to about 2 per cent sets upon cooling to a firm gel solidifying at about 35° to about 50° C. and melting at about 90° to about 100° C. In view of the fact that agar is derived from biological sources, there are numerous disadvantages associated with its use. First, use of different species and genera of algae to produce agar results in an agar solid phase differing in gel strength, elasticity, syneresis, viscosity, transparency, ash content and content of impurities. Changes in environment, temperature, and light, for example, are known to affect the type of agar produced by various algae. Thus, variations in the properties of agar due to the type of algae and the season of the year are observed.

In the use of agar in a culture growing medium, it is required that an artisan go through a standard set of steps to render the agar soluble, that is, the agar must be heated in admixture with a nutrient or mixtures of nutrients to a temperature ranging from about 90 to 100° C. and then the admixture must be cooled to about 35 to 50° C. to produce the gel-like material. After standing the agar-containing medium will tend to dry out and of course another heating and cooling step is required to cause the agar to again reincorporate any additional water to form the gel-like material.

One of the serious disadvantages of agar is that it does not adhere well to the inner surfaces of the Petri-dish. A sharp blow to an inverted dish will, in many instances, dislodge the agar or sufficiently loosen it from the dish so that organisms can penetrate and grow in the voids thus created. Various devices have been reported in the literature, such as the disposable culturing device disclosed in U.S. Patent Specification No. 2,874,091, wherein the media is indicated as clinging to the dish. Although this device employs a culturing dish composed of a thermoplastic material, i.e., a styrene plastic, the actual culturing media disclosed are various well-known forms of agar. While the agar media may adhere better to the plastic than to glass, they still cannot withstand severe shock as might be encountered in shipping and handling with the resultant dislodgement of the media.

A further disadvantage of the agar media currently in use is their inability to absorb fairly large quantities of liquid and thus act as a filter for microorganisms. This filtering feature would be of particular advantage in a culturing medium system where a significant

amount, e.g. 5 to 20 cc of fluid sample, such as drinking water, beer, body fluids and the like could merely be poured onto the medium. The liquid portion would be absorbed into the medium while the microorganisms remain on the surface for propagation. The surface growth would greatly facilitate counting and isolation of the microorganisms.

Various substitutes for agar have been proposed over the past few years but none has been perfected to the point where it has been a commercially acceptable replacement. In U.S. Patent Specification No. 3,247,078 a process is disclosed for the propagation of microorganisms using a matrix of poly-(ethylene oxide). However, in this process the polymer is dehydrated and subdivided into particles by normal grinding operations. The culture growing medium is then prepared by addition of water and a nutrient or nutrients. The resulting propagating medium is not intimately bonded to its container and hence can be dislodged in the same manner as agar. Thus, it is not desirable to ship plates which are ready to use but rather, to ship the ground polymer which then must be rehydrated prior to use.

It is therefore an object of this invention to provide an improved microbiological testing device wherein many of the disadvantages associated with presently employed devices are eliminated or greatly minimized. Another object of this invention is to provide an improved, disposable microbiological testing device which can be pre-sterilized and shipped with no danger of the media separating from the container. A further object of this invention is to provide an improved microbiological testing device which can be stored or shipped in the dehydrated form and rehydrated merely by the addition of water or other liquids. Another object of this invention is to provide an improved microbiological testing device which is capable of filtering microorganisms from fluids so that they remain on the surface. A still further object of this invention is to provide a microbiological testing device which enhances microorganism identification with a minimum of swarming. Another object of this invention is to provide a process for the preparation of the improved microbiological testing device of this invention. These and other objects will readily become apparent to those skilled in the art in the light of the teachings herein set forth.

In a broad aspect, the present invention is directed to an improved microbiological testing device and to a process for its preparation. The device of this invention is comprised of a container, such as a Petri-dish, and a culturing medium system contained in and integrally bonded to the container, the culturing medium system, as hereinafter defined, is capable of being dehydrated and rehydrated to its original form in the container without substantial loss of bonding to the container itself. It is also capable of filtering microorganisms from fluids so that they remain on the medium surface. Moreover, the medium system enhances microorganism propagation with a minimum of swarming.

In practice, the materials which have been found to be useful as culturing medium systems in the device of this invention are transparent, substantially insoluble hydrophilic gels having the characteristic properties hereinbefore enumerated. Illustrative hydrophilic gels which are useful in the culturing device of this invention are those prepared from the following starting materials: poly-(ethylene oxide), polyvinyl pyrrolidone, polyvinyl alcohol, polyacrylamide, polyvinylmethyl ether, copolymers of maleic anhydride and ethylene, and copolymers of maleic anhydride and vinylmethyl ether.

Also suitable for use in the practice of the present invention are hydrogels of crosslinked hydrophilic polymers comprised of copolymers of (1) a major amount of a polymerizable monoester of acrylic acid or methacrylic acid which monoester has a single olefinic double bond, and (2) a minor amount of a polymerizable diester of one of said acids, which diester has at least 2 olefinic double bonds. Further description of this type of copolymer can be found in U.S. Patent Specification No. 3,220,960.

Insoluble hydrophilic gels especially amenable for use in the present invention because they possess the ability to incorporate very large amounts of water in the order of 10 to 100 times their dry weight include water insoluble poly(ethylene oxide), water insoluble copolymers of ethylene oxide and propylene oxide, and water insoluble alkyl substituted phenyl ethers of ethylene oxide polymers wherein the alkyl groups may be methyl and/or butyl. These polymers, in addition to possessing the ability to incorporate large amounts of water, are insoluble in water irrespective of temperature, will retain liquids, solutions and suspensions and will form gel-like products.

The water insoluble polymers mentioned immediately above may be conveniently prepared by utilizing ionizing radiation. As used herein, the term "ionizing radiation" includes that radiation which has sufficient energy to cause electronic excitation and/or ionization in the polymer molecules and solvent molecules (where a solvent is employed) but which does not have sufficient energy to affect the nuclei of the constituent atoms. Convenient sources of suitable ionizing radiation are gamma ray producing radio-

active isotopes such as Co^{60} and Cs^{137}, spent nuclear fuel elements, X-rays, such as those produced by conventional X-ray machines, and electrons produced by such means as Van de Graaff accelerators, linear electron accelerators, resonance transformers and the like. Suitable ionizing radiation for use in the present invention will generally have an energy level in the range from about 0.05 mev. to about 20 mev.

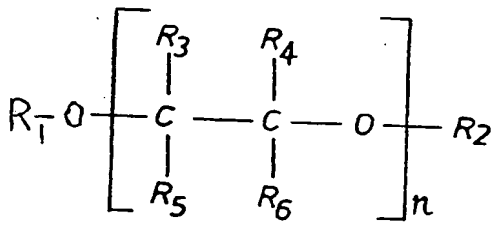
The irradiation of the non-crosslinked (and usually water soluble) polymers can be carried out in the solid phase or in solution. Solid polymers can be irradiated in the air, in a vacuum, or under various gaseous atmospheres, while irradiation in solution can be carried out with the polymer dissolved in water, in conventional organic solvents, or in mixtures of water and water miscible organic solvents. Any convenient method can be used to bring the solid polymer or polymer solution into contact with the ionizing radiation. Suitable methods are well known and understood by those skilled in the art.

The exact amount of ionizing radiation to which the polymers must be subjected depends on a number of variables. In general, when irradiation is carried out at relatively low rates and in the presence of free radical scavengers, such as oxygen, extremely high total doses are required to produce the water insoluble materials useful in this invention. On the other hand, when the irradiation is carried out under conditions which favor the relatively long existence of the free radicals produced, as for example, when the irradiation is carried out with a high dose rate, in the absence of oxygen, or in solution where oxygen is rapidly used up, the formation of water insoluble polymeric materials useful in this invention takes place readily. The preferred method for producing the water insoluble polymers useful in this invention which have the highest water absorptive capacity is to carry out the irradiation on an aqueous solution of the polymer while employing ionizing radiation having an energy level in the range of about 0.50 mev. to about 20 mev. at a total dose of between about 0.05 and 10 megarads.

The terms "insoluble" or "insolubilization" as employed throughout the specification are utilized herein to define the formation of a gel which is essentially solid.

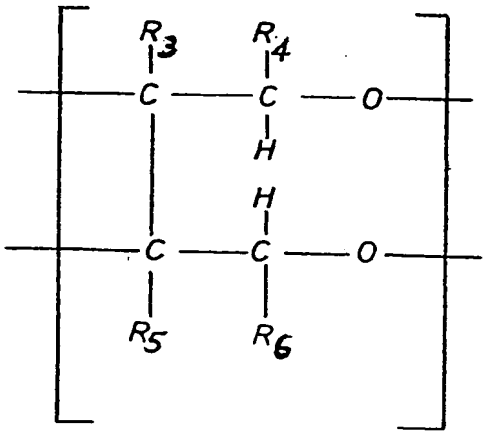
The insolubilization can be effected by a wide variety of methods and includes, but is not limited to, ionizing and non-ionizing radiation, and chemical cross-linking through covalent and ionic bonding.

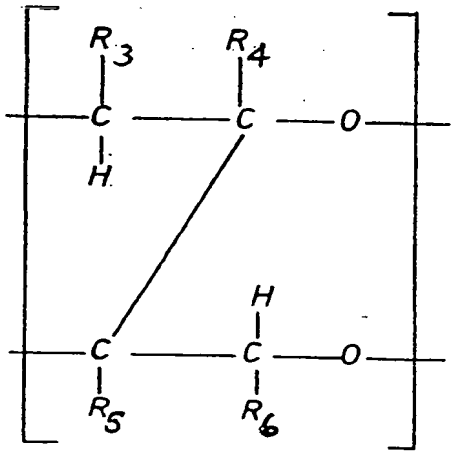
In one preferred aspect, the culturing medium system of this invention is comprised of a hydrophilic, polymeric gel of at least one polymer of the formula:



which has been cross-linked and wherein R_1 and R_2 are each hydrogen, an alkyl radical or an alkyl substituted aryl radical, and wherein R_3 is hydrogen when R_4 is hydrogen, or a methyl, phenyl or vinyl radical, R_4 is hydrogen when R_3 is hydrogen, or a methyl, phenyl or vinyl radical, R_5 is hydrogen when R_6 is hydrogen, or a methyl, phenyl or vinyl radical, and R_6 is hydrogen when R_5 is hydrogen, or a methyl, phenyl or vinyl radical, and n is greater than one.

The hydrophilic gels are polymeric compounds containing at least one of the structural units shown below:



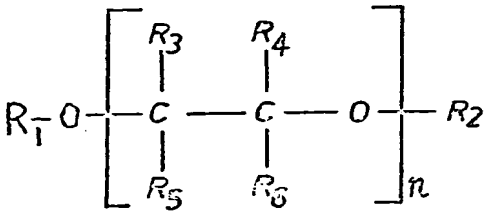


wherein R_3 is hydrogen when R_4 is hydrogen, or a methyl, phenyl or vinyl radical; R_4 is hydrogen when R_3 is hydrogen, or a methyl, phenyl or vinyl radical; R_5 is hydrogen when R_6 is hydrogen, or a methyl, phenyl or vinyl radical; and wherein R_4 is hydrogen when R_5 is hydrogen, or a methyl, phenyl or vinyl radical.

These hydrophilic polymeric gel compounds may include carbon-to-carbon cross-linking between straight chain carbon atoms and the carbon atoms of branch chain methyl groups and in addition a very minor and insignificant number of bonds may include two oxygen atoms linking the carbon atoms.

The polymeric gel compounds may contain cross-linking bonds present as inter-molecular bonds (e.g. between two different molecules) and intramolecular bonds (e.g. between carbon atoms of the same molecule), and combinations of intra and intermolecular cross-linking bonds.

The process for producing these polymeric gels from poly(ethylene oxide) comprises preparing a homogeneous water solution of at least one of the water soluble compounds selected from the following class of compounds:



wherein R_1 and R_2 are each hydrogen, an alkyl radical or an alkyl substituted aryl radical, R_3 is hydrogen when R_4 is hydrogen, or a methyl, phenyl, or vinyl radical, R_4 is hydrogen when R_3 is hydrogen, or a methyl, phenyl or vinyl radical, R_5 is hydrogen when R_6 is hydrogen, or a methyl, phenyl or vinyl radical, R_6 is hydrogen when R_5 is hydrogen, or a methyl, phenyl or vinyl radical, and n is greater than one; and submitting the solution to ionizing radiation for a period of time sufficient to cause insolubilization of the polymer.

The preferred starting materials for preparing the culturing medium system for use in the devices of the present invention are ethylene oxide polymers which have a reduced viscosity value of at least 0.5 and upwards to 75, and higher; or an aqueous viscosity at 25° C. of from 225 centipoises, as measured at a 5 weight per cent concentration, to 12,000 centipoises, and higher, as measured at a 1 weight per cent solution. The polymers which are particularly useful are the ethylene oxide homopolymers and ethylene oxide copolymers or terpolymers comprised of at least 50 weight per cent of ethylene oxide in copolymerized form with up to 50 weight per cent of at least one other lower olefin oxide such as propylene oxide, butylene oxide or styrene oxide.

The reduced viscosity is measured by the following method: Transfer 100 ml of acetonitrile to an 8-oz., round, screw-cap bottle. With constant stirring introduce into the bottle, 0.200 gm of the polymer weighed to the nearest mg. Line the screw-cap of the bottle with a piece of aluminum foil, carefully place the cap on the bottle, and tighten securely. Place the bottle on a suitable can roller with 6-inch (I.D.) rollers, and allow it to roll for 16±0.5 hr. Remove the bottle from the roller, and filter the solution by pressure through a coarse, sintered glass filter. Determine the time in seconds required for the sample solution to pass through a calibrated Ubbelohde suspended-level viscometer at 30+0.01° C. Use a suitable stopwatch with a 10-second dial graduated in 0.1 second units, accurate to within 0.1 per cent when tested over a 60-minute period. Record the time required. Determine and record the time in seconds required for the acetonitrile to pass through the viscometer.

Calculation

$$AS - \frac{F}{AS} = AC$$

$$SS - \frac{F}{SS} = SC$$

$$\frac{SC - AC}{AC} = SV$$

$$\frac{SV}{K} = RV$$

F = viscometer correction
AS = seconds, required for acetonitrile
AC = seconds, corrected, required for the acetonitrile
SS = seconds required for polymer solution
SC = seconds, corrected, required for solution
SV = specific viscosity
RV = reduced viscosity
K = concentration, gm sample per 100 ml acetonitrile

The following table illustrates the relation between the average molecular weight of poly(ethylene oxide), reduced viscosity and bulk viscosity of solutions thereof.

Wt. — % polymer in acetonitrile	Reduced Viscosity	Approximage Average molecular weight	Bulk viscosity aqueous solution at 25°C.
0.2	1.5	150,000	200 cps (5 wt.—% soln.)
0.2	60	10,000,000	7000—9000 cps (1 wt.—% soln.)

The terms "aqueous viscosity" or "bulk viscosity" as employed herein refer to the viscosity of the stated concentration of polymer in water, as measured on a Model RVF Broofield Viscometer using a No. 1 spindle operated at 2 revolutions per minute, unless otherwise stated. The viscosity is measured at ambient room temperatures, that is, about 24° C.

It should be noted that the instant invention is not limited to the use of the starting materials listed above, but includes for example copolymers of one or more of either the aforementioned compounds. For example, copolymers of ethylene oxide and minor or major amounts of other alkylene oxides can also be used.

As previously indicated, an outstanding feature of this invention is that the culturing medium system is integrally bonded to the culturing container. The bond is so strong that in most instances the dish or container will break before the medium can be dislodged. In practice, the binding of the culture medium to the container is accomplished simultaneously with the irradiation or insolubilization of the polymer solutions.

It is therefore critical to the invention that the radiation dose received at the interfaces between the dish and the polymer be of sufficient magnitude to integrally bond the two as well as to insolubilize the polymer. However, a complicating factor is that for a monenergetic beam, i.e. one in which all the electrons carry the same energy, the radiation dosage varies with the depth of penetration. Firstly, the dosage at the solution surface is about 60 per cent of the maximum. The maximum dosage occurs at a point about one-third through the total penetration range, and then declines. Secondly, the depth at which the ionization is equal to that occurring at the surface is equal to about two-thirds of the total range. Thus, if all parts of the polymeric solution are to receive the same minimum dosage, the useful penetration is two-thirds of the total. Since a 1 million electron-volt (MeV) electron beam has a maximum penetration of about 0.5 crs/cm² the useful penetration is about 0.33 crs/cm². Therefore, the insolubilization and bonding of the hydrogel to the sides and bottom of the dish must be done under conditions that insure a proper dosage to all portions of the material.

Table I, below, sets forth the range and thickness of solutions of poly(ethylene oxide) that can be irradiated with electrons of different energies. The "optimum thickness," assuming a density of 1, is that thickness which will give the same dose to the top and bottom of the solution and will result in satisfactory bonding of the gel to the dish.

TABLE I

Energy	Max. range cm	Usable Thickness (cm)	Dose(a) Top	Dose(a) Bottom	Optimum Thickness (cm)	Dose (b) Top	Dose (b) Bottom
.5 MeV	.24	0.18	1	0.4	0.15	1	1
1.0 MeV	0.45	0.37	1	0.4	0.29	1	1
2.0 MeV	0.9	0.73	1	0.4	0.58	1	1
3.0 MeV	1.32	1.07	1	0.4	0.87	1	1

(a) If the dose at the surface is 1 unit, the dose at the bottom is only 0.4 of the surface dose.

(b) Equal doses at top and bottom.

Hence, for example, by plotting a depth-dose curve showing the percent energy deposited versus the thickness for a 1 MeV electron, it was observed that an 85 milliliter Petri dish required approximately 16.4 cubic centimeters of polymer solution to get an equal entrance and exit dose. Accordingly, it is important that both insolubilization and bonding be achieved through the proper choice of irradiation dosages. In practice, it has been found that the optimum results are obtained when the irradiation conditions are chosen such that the surface of the polymer solution and the interface between the solution and dish receive approximately the same dosage.

In practice, the microbiological testing devices of this invention can be prepared with or without nutrients. In some instances it may be desirable to insolubilize the polymeric solutions and add the nutrients just prior to use. In other instances the nutrients can be incorporated into the hydrogel immediately after its preparation. The resulting plate is then ready for use.

As employed throughout the specification and appended claims, the term "culturing medium system" is intended to encompass the culturing medium with and without nutrients. Thus, for example, the term can encompass the polymeric matrix alone, i.e. the insoluble hydrophilic gel, or the polymeric matrix having absorbed therein one or more known nutrients.

The term "nutrient" while well established in the art to indicate the material which provides nourishment to bacteria, fungi and tissues of more complex organisms, is specificially defined herein to include a wide variety of particular nutritional, diagnostic and/or growth factor species in a dispersing medium such as water and/or organic liquids.

Nutrients in general consist of aqueous solutions or dispersions containing any one or combination of a variety of nutritional species. The particular nutritional species may exist as true solutes in the dispersing medium and/or as colloidal suspensions. In the present invention the term "nutrient" is defined to include nutritional species in organic dispersing liquids in addition to aqueous dispersing liquids.

The nutritional species include the broad classes, e.g. proteins and their degradation products, nucleic acid and the degradation products thereof, carbohydrates, fats, vitamins, hormones, diagnostic agents, dyes, and macro and micro inorganic nutrients.

More particularly the term nutrient includes the following nutritional species; the proteins ranging from the polypeptides such as peptones or proteoses to the amino acids, respectively present in the dispersing medium as colloids in the case of some polypeptides or true solutes in the case of amino amino acids; nucleic acids ranging from the highest molecular weight polymers, which may be present as colloids, through polynucleotides or oligo nucleotides to monomeric nucleotides normally present as true solutes; carbohydrates ranging from colloidal polysaccharides such as starch, dextrin and glycogen through the oligo saccharides to simple solutes of mono and disaccharides such as glucose and sucrose; fats and lipiodal material such as fat-protein complexes as either colloidal materials or as true solutes; growth factors such as the vitamins, generally present at low concentrations such as .01 weight per cent or less; small amounts of hormonal growth factors; diagnostic agents including antibiotics such as penicillin, germicides and antiseptics such as phenols; dyes such as eosin and methylene blue; macro nutrients such as calcium, sodium, potassium, sulfur, sulphates, nitrogen, nitrates, phosphorus, phosphates and iron; and micro nutrients or trace elements such as cobalt, zinc, iodine, vanadium, boron, manganese and copper.

In addition to the fact that the culturing medium system is integrally bonded to the container and that the nutrients can be incorporated at most any stage, a further advantage of the testing device is that the hydrophilic gel can be dehydrated and rehydrated. This feature is particularly attractive if one desires to store sterile plates for long periods of time or wishes to add relatively large amounts of liquids containing the organisms to be cultured. For example, plates can be conveniently prepared for analysis of raw water, beer, wine, body fluids, and the like, by merely pouring the liquid onto the partially or completely dehydrated culturing medium system and allowing the liquid to be absorbed. The feature is also important where few organisms are present in the liquid. In contrast to streaking agar with a small amount of liquid, relatively large quantities of liquid can be poured onto the dehydrated medium. This assures the presence of more organisms and the resulting accuracy of their identification.

The culturing medium system with or without nutrients can be dehydrated by any of several methods. For example, the dish containing the insolubilized hydrogel can be passed through an infrared drying system or exposed to hot air. In general, the hydrogel can be essentially completely dehydrated and still remain firmly bonded to the dish when rehydrated. The degree to which the hydrogel is dehydrated, will, of course, be determined by a variety of considerations, such as the amount of liquid to be added, the type of organism being cultured and the like.

As previously indicated, the insolubilized hydrogel acts as a filter and retains the organisms on its surface. This aspect is particularly attractive for microbiological analysis

of liquids such as water, body fluids, and the like, which can be poured directly onto and absorbed into the hydrogen. Hence, coupled with the fact that the hydrogel is integrally bonded to the dish and no liquid sample can penetrate between the hydrogen and the dish walls, the filtering feature assures that the organisms are concentrated on the gel surface.

It has also been observed that the culturing medium system of this invention can minimize swarming of motile organisms. For a given number of colonies grown on agar and the hydrogels of this invention, it has been observed that those grown on the latter are isolated and can easily be separated. This feature avoids the necessity, in many instances, of admixing antibiotics or other ingredients with the nutrient to suppress undesirable colonies which may obscure or interfere with the organism in question. Moreover, when swarming is minimized the organisms can easily be isolated.

The following examples are illustrative:

Example I.

Preparation of Hydrophilic Gel Bonded to Petri Dishes.

Into standard Petri dishes (85 millimeter diameter) which had been thoroughly cleaned by subjecting them to a Corona discharge were added 15 milliliters of a 3.4 weight per cent aqueous solution of coagulant grade (poly-(ethylene oxide). Prior to pouring, the viscosity of the solution was adjusted to 500—1000 centipoises from its original viscosity by mechanical shearing. Any impurities were removed from the solution by microporous membrane filtration and the pH adjusted to 7.0.

The dishes containing the poly(ethylene oxide) solution were then irradiated, with a beam of high energy electrons generated from a Van de Graaff accelerator, to an absorbed dose of about 0.7 Megarads during approximately 6 seconds. After irradiation, the dishes were passed through an infrared drying system and dehydrated so that the remaining gel weighed approximately 8 grams. Both before and after the dehydration step, it was observed that the gel was firmly bonded to the dish and attempts to dislodge it were unsuccessful.

A sterile nutrient, i.e., Tryptic Soy Broth, was then added to a portion of the dishes. The concentration of the nutrient broth was adjusted so that the final concentration was that commonly employed for culturing microorganisms. After the nutrient was absorbed the dishes were ready for inoculation.

Tryptic Soy Broth was then added to the remainder of the dishes and allowed to soak in for 4 to 6 hours. The gel was then further dehydrated by hot air to essentially complete dryness. Thereafter, the Petri dish was packaged in polyethylene film and sterilized with ethylene oxide using standard techniques.

Portions of the dishes were converted into usable form by the addition of the appropriate amount of sterile water. These plates were used for standard microbiological procedures.

Another portion of the dishes were rehydrated with a sample of raw water. After the water had been incorporated, the plates were incubated overnight at 37.5°C. Well isolated colonies of the organisms commonly found in raw water were observed.

Examples 2 to 8.

In a manner similar to that employed in the previous example, Petri dishes are prepared containing other insoluble hydrogels integrally bonded to the bottom and sides of the dishes. The polymers employed and other pertinent data are set forth in Table I below:

TABLE I

Example	Polymer	Solution Conc. Wt. %	pH	Irradiation Dose in MRads	Appearance of Gels
2	Polyvinyl pyrrolidone	4	3.6 and 7.0	0.7—2.1	Satisfactory
3	Polyvinyl alcohol	3	2.5 and 6.2	0.7—2.1	Satisfactory
4	Polyacrylamide	3 and 2	4.0 5.4, 7.0	0.7—2.1	Satisfactory
5	Polyvinyl-methyl ether	2	4.3 2.5, 12.0	0.7—1.5	Satisfactory
6	Polyacrylic acid	4	2.4 4.0	0.7—2.1	Satisfactory
7	Copolymer of maleic anhydride and ethylene	2	2.4	0.7—2.0	Satisfactory
8	Copolymer of maleic anhydride and vinyl methyl ether	2.5	2.0	0.7—2.1	Satisfactory

Attempts to remove the insoluble hydrogels from the Petri dishes by a sharp blow of the inverted dishes on a hard surface, were unsuccessful. Further efforts to remove the gel by flexing the dish itself resulted in shattering of the dish with the hydrogel still intact.

Example 9.

In order to compare the growth promoting ability of plates prepared with nutrient-containing hydrophilic gels of this invention with those prepared from agar and the same nutrients, a series of Petri dishes containing coagulant grade poly(ethylene oxide) insolubilized as in Example 1, and nutrients and agar and nutrients were prepared and inoculated with *Escherichia coli*.

The poly(ethylene oxide) plates were prepared in accordance with the procedure described in Example 1 and the gel dehydrated to 7.0 or 8.0 grams per plate as indicated below. To each plate was added the indicated quantity of Tryptic Soy Broth solution, the concentration of which was adjusted so that when the solution was in equilibrium with the gel, it represented an agar plate containing nutrients in the concentration commonly used in culturing microorganisms.

Table II below sets forth the amount of dehydrated gel, the milliliters of Tryptic Soy Broth solution employed and its concentration.

TABLE II

Series	Grams of Dehydrated Gel	Milliliters TSB Solution	Factor of Normal Conc. of TSB Solution
1	7.0	3.0	3.33
2	7.0	5.0	2.40
3	8.0	3.0	3.68
4	8.0	5.0	2.60
5	7.0	3.0	3.33
6	7.0	5.0	2.40

The agar plates were prepared by accepted techniques and consisted of suspending 15.0 grams of Tryptic Soy Broth in 500 milliliters of distilled water to which 7.5 grams of "Difco" Bacto Agar had been added. ("Difco" is a Registered Trade Mark). This suspension was then heated to boiling until all the agar was dissolved and then steam sterilized for 15 minutes at 121°C. Plates were then poured in the same volume per dish as the poly(ethylene oxide) series.

Both series of plates were inoculated with *E. coli.* in accordance with the following procedure:

A twenty-four hour Tryptic Soy Broth culture of *E. coli.* was serially diluted from 10^{-1} to 10^{-7} by transferring 1.0 milliliter in 9.0 milliliters sterile saline dilution blanks. Thereafter 0.1 milliliters of the 10^{-6} dilution was aseptically transferred to each of twenty plates of the poly(ethylene oxide) and to the agar plates. Similarly 0.1 milliliters of the 10^{-7} dilution was aseptically transferred to each of 20 plates of the poly(ethylene oxide) and agar. 0.1 Milliliters of the 10^{-6} dilution actually represents a 10^{-7} inoculation, while 0.1 milliliters of 10^{-7} dilution represents a 10^{-8} inoculation.

These organisms were distributed over the surface of the poly(ethylene oxide) and agar plates by streaking with a sterile bent glass rod. This insured a uniform distribution of inoculated organisms on the surfaces resulting in the growth of countable isolated colonies. The plates were then incubated at 37.5°C for 24 hours and colony counts taken through the use of a Quebec Colony Counter.

Table III below sets forth the average counts of ten plates for each series of poly(ethylene oxide) and agar at 10^{-7} dilution and ten plates for each series at 10^{-8} dilution.

TABLE III

Colony Counts

	Dilution	Series 1	Series 2	Series 3	Series 4	Series 5	Series 6	Agar Series
Aver.	10^{-7}	219	214	229	203	212	212	198
Aver.	10^{-8}	25	21	24	23	25	20	12

From the foregoing data it was evident that the series of poly(ethylene oxide) plates show better growth promoting ability as far as enumeration of organisms than does the series of agar plates.

Example 10.

A nutrient solution of Tryptic Soy Broth of proper concentration was prepared and sterilized by steam sterilization. Thereafter, the flasks containing the solution were cooled to ambient temperature and a quantity of untreated lake water added to the sterile solutions. The flasks were then swirled for a sufficient time to allow for uniform dispersion of the lake water and the contaminating organisms contained therein.

A series of plates containing 8 grams of poly(ethylene oxide) gel prepared in accordance with the process of Example 1 were each charged with 7.0 milliliters of the above solution using sterile techniques. The solution was distributed over the entire surface of the gel by gently tilting and rotating the plates. Thereafter the plates were permitted to remain at room temperature until all of the solution had been absorded into the gel. The plates were then incubated at 37.5°C. for 24 hours and the results observed. Each of the plates exhibited a wide variety of contaminating organisms which were isolated and occurred only on the surface of the gel. No subsurface organisms were detected, indicating that the gel retained all the contaminating organisms on the surface.

In a similar manner, a quantity of sterile Tryptic Soy Broth is contaminated with a quantity of 10^{-7} dilution of *E. coli.* 7.0 Milliliters of this solution is added to the dehydrated poly(ethylene oxide) and the plates incubated as above. All the organisms are retained on the surface and no subsurface growth detected.

Example 11.

In order to demonstrate the inhibition of swarming of *Proteus* organisms, experiments were performed comparing the degree of swarming on plates prepared with coagulant grade poly(ethylene oxide) with those prepared from agar. The poly(ethylene oxide) plates were prepared by the addition of 1.0 milliliter of Tryptic Soy Broth to partially dehydrated poly(ethylene oxide) plates prepared in accordance with Example 1. The gel weight prior to rehydration was 8.0 grams. The concentration of the Tryptic Soy Broth employed was adjusted so that the resultant plate contained the standard concentration of nutrients. Agar plates

containing the same concentration of Tryptic Soy Broth were prepared by adding 1.5 per cent agar to the nutrient solution.

To each of 20 poly(ethylene oxide) plates and 20 agar plates was added 0.1 milliliters of 10^{-6} dilution of a 24 hour culture of *Proteus mirabilis*. This represented a 10^{-8} dilution. The inoculum was uniformly spread over the surface of the gel and the agar through the use of a sterile, bent glass rod. These plates were then incubated for 24 hours at 37.5°C. The agar plates showed a heavy film of growth over the entire surface of the plate resulting from a great degree of swarming of the Proteus organisms. No isolated colonies were observed and hence the performance of a colony count was impossible. The results obtained on the poly(ethylene oxide) plates indicated excellent isolation of colonies resulting from the fact that swarming had successfully been inhibited. Approximately 100—200 isolated, well defined and non-swarminng colonies were observed on each of the plates containing the poly(ethylene oxide). In contrast to the agar plates, colony counts were easily performed on these plates.

Example 12.

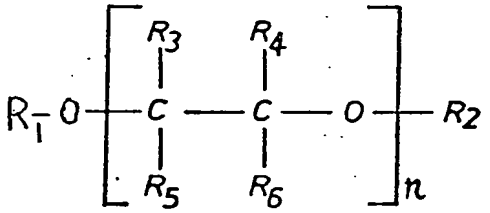
In order to evaluate the growth promoting ability of other insoluble hydrogels hereinbefore disclosed, Petri dishes are prepared containing the various insolubilized polymeric materials, and inoculated with different cultures. The hydrogels are prepared in accordance with the procedure employed in Example 1 from polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl methyl ether, polyacrylamide, copolymer of maleic anhydride and ethylene, copolymer of maleic anhydride and vinylmethyl ether and the hydrogel of U.S. Patent Specification No. 3,220,960.

For comparison purposes, plates containing agar and the same nutrients are employed. The plates containing the insolubilized polymeric materials show better growth promoting ability with regard to enumeration of organisms. Similar experiments also indicate that these materials possess the filtration properties and the ability to minimize swarming.

WHAT WE CLAIM IS:—

1. Microbiological testing device comprising in combination, a culturing container and a culturing medium system integrally bonded to said container, said medium system comprising a transparent substantially insoluble, hydrophilic gel, said gel being characterized in that (a) it can be dehydrated and rehydrated in said container without substantial loss of bonding to said container, (b) it can filter and retain microorganisms on its surface while absorbing fluids in which said microorganisms are present, and (c) it can minimize swarming of motile organisms.

2. A testing device according to claim 1 wherein said hydrophilic gel comprises at least one polymer of the formula:



which has been cross-linked, and wherein R_1 and R_2 each represent hydrogen, an alkyl radical or an alkyl substituted aryl radical, and R_3, R_4, R_5 and R_6 each represent hydrogen or a methyl, phenyl or vinyl radical, with the proviso that at least one of R_3 and R_4 and at least one of R_5 and R_6 are always hydrogen and n is greater than one.

3. A testing device according to claim 1 wherein said hydrophilic gel comprises cross-linked poly (ethylene oxide).

4. A testing device according to claim 1 wherein said hydrophilic gel comprises cross-linked polyvinyl pyrrolidone.

5. A testing device according to claim 1 wherein said hydrophilic gel comprises cross-linked polyvinyl alcohol.

6. A testing device according to claim 1 wherein said hydrophilic gel comprises cross-linked polyacrylamide.

7. A testing device according to claim 1 wherein said hydrophilic gel comprises cross-linked polyvinyl-methyl ether.

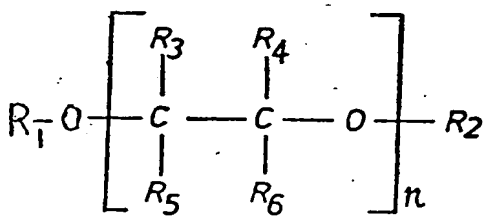
8. A testing device according to claim 1 wherein said hydrophilic gel comprises a cross-linked copolymer of maleic anhydride and ethylene.

9. A testing device according to claim 1 wherein said hydrophilic gel comprises a cross-linked copolymer of maleic anhydride and vinylmethyl ether.

10. A testing device according to claim 1 wherein said hydrophilic gel comprises a cross-linked copolymer of (1) a major amount of a polymerizable monoester of acrylic acid having a single olefinic double bond and (2) a minor amount of diester of acrylic acid, said diester having at least 2 olefinic double bonds.

11. A testing device according to any one of the preceding claims wherein said culturing medium system contains nutrients.

12. A process for the preparation of a microbiological testing device as claimed in Claim 1 which process comprises the steps of (a) adding to a culturing container an aqueous solution of a polymer of the formula:



wherein R_1 and R_2 each represent hydrogen, an alkyl radical or an alkyl substituted aryl radical, and R_3, R_4, R_5 and R_6 each represent hydrogen or a methyl, phenyl or vinyl radical with the proviso that at least one of R_3 and R_4 and at least one of R_5 and R_6 are always hydrogen and n is greater than one, and (b) exposing said solution to ionising radiation for a period of time sufficient to cause bonding of said polymer to said container.

13. A process for the preparation of a microbiological testing device as claimed in claim 1, which process comprises the steps of (a) adding to a culturing container an aqueous solution of a polymer comprising poly-(ethylene oxide), polyvinyl pyrrolidone, polyvinyl alcohol, polyacrylamide, polyvinyl methyl ether, a copolymer of maleic anhydride and ethylene, or a copolymer of maleic anhydride and vinyl methyl ether, and (b) exposing said container and solution to a beam of high energy electrons to cross-link said polymer and bond it to said container.

14. A process according to claim 13 wherein said cross-linked polymer is subsequently dehydrated.

15. A process according to claim 13 or claim 14 wherein nutrients are added to said cross-linked polymer.

16. A process according to claim 15 wherein said culturing medium system is subsequently dehydrated.

17. A process for the preparation of a microbiological testing device, as claimed in claim 1 which process comprises the steps of: (a) adding to a culturing container an aqueous solution of a copolymer derived from (1) a major amount of a polymerizable monoester of acrylic acid having a single olefinic double bond and (2) a minor amount of a diester of acrylic acid, said diester having at least 2 olefinic double bonds and (b) exposing said container and solution to a beam of high energy electrons to cross-link said copolymer and bond it to said container.

18. A process according to claim 17 wherein said cross-linked copolymer is subsequently dehydrated.

19. A process according to claim 17 or claim 18 wherein said nutrients are added to said cross-linked copolymer.

20. A process according to claim 19 wherein said culturing medium system is subsequently dehydrated.

21. A process for the preparation of a microbiological testing device according to claim 12 substantially as described with reference to the Examples.

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